PHYSIOLOGY OF INSECT DIAPAUSE. III. THE PROTHORACIC GLANDS IN THE CECROPIA SILKWORM, WITH SPECIAL REFERENCE TO THEIR SIGNIFICANCE IN EMBRYONIC AND POSTEMBRYONIC DEVELOPMENT

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In recent years it has become increasingly evident that insect metamorphosis is under the control of an array of factors generally presumed to be hormonal in character. Interest has heretofore centered around the endocrine functions of the brain, the corpora cardiaca, and the corpora allata, including the "ring gland" of Diptera. Since these organs are usually situated in the head, it has not been easy to account for the fact that a thoracic center exercises important control over the embryonic and postembryonic development of many species (Richards and Miller, 1937; Richards, 1937).

The existence of a thoracic "differentiation center" seems, indeed, to be a distinctive feature of the embryology of all insects (Schnetter, 1934). As will become evident subsequently, the position of this embryonic center is of special interest. According to Richards and Miller (page 35), it "extends from the second gnathal (maxillary) segment to the second thoracic segment with its midpoint in the anterior half of the presumptive prothorax."

In the case of postembryonic development the evidence in favor of the existence of a thoracic "differentiation center" has been especially prominent in studies of the Lepidoptera. Here the thorax is believed to preside over moulting (Fukuda, 1940b), pupation (Bodenstein, 1938; Bounhiol, 1938; Fukuda, 1940a), and adult development (Hachlow, 1931; Bounhiol, 1938; Fukuda, 1941; Williams, 1947). The thorax has not been implicated, to date, in the postembryonic development of the Diptera, Orthoptera, or Hemiptera, but according to Geigy and Ochsé (1940) the thorax or first abdominal segment controls pupation in the megalopteran, *Sialis*.

These observations take on renewed interest as a result of Fukuda's studies of the commercial silkworm, *Bombyx mori*, where pupation and adult formation were found to be dependent on the function of a previously overlooked organ, the "prothoracic glands." For the first time the identity of a thoracic differentiation center was thereby established. The prothoracic glands therefore merit special attention, for homologous structures may be widely distributed among insects and conceivably act as the thoracic center that is known to control embryonic and, in certain species, postembryonic development. For this reason the existing literature pertaining to the prothoracic glands will be briefly reviewed.

Prothoracic glands were first described by Toyama (1902) in his study of the embryology of *Bombyx mori*. They arise early in embryonic development as

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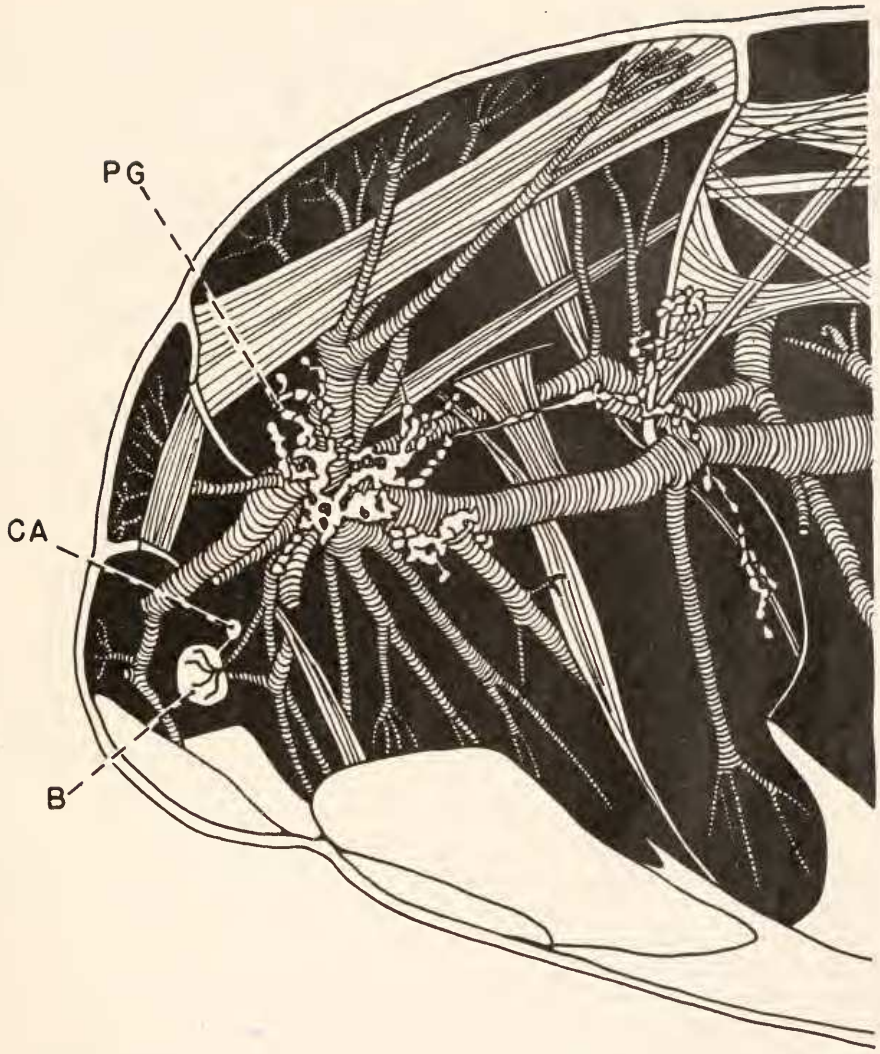

FIGURE 1. The prothoracic gland in the right half of the thorax of a diapausing Cecropia pupa. Fat body and numerous other tissues have been cleared away: B, Brain; CA, Corpus allatum and Corpus cardiacum; PG, Prothoracic glands.

epithelial invaginations of the lateral part of the second maxillary segment, from which they extend posteriorly into the prothorax. This location may, to advantage, be compared with that of the "differentiation center" in embryonic insects, described above.

Although Toyama's discovery has been generally overlooked, it is to his credit that he recognized the prothoracic glands not only as being hitherto unknown, but also as being glandular organs. They were named "hypostigmatic glands," an unfortunate designation, since the term had previously been applied to wholly different

tissues in the Lepidoptera (Verson and Bisson, 1891). Twenty-eight years elapsed before the glands were next mentioned in the literature. Ke (1930) then named the organs "prothoracic glands" and described certain variations in their branching. In a paper to be published in the near future, Lee (1948) will present a morphological study of the prothoracic glands in lepidopterous larvae, with special reference to their innervation.

Physiological function for the prothoracic glands was first discovered by Fukuda (1940a, 1940b, 1941) in a series of studies reviewed elsewhere (Williams, 1948). Most recently, the function of the prothoracic glands has been considered in relation to adult development of several species of silkworms (Williams, 1947, 1948).

Morphology and Histology

In my experience the prothoracic glands can be demonstrated most satisfactorily by gross dissections of fresh material submerged in insect Ringer's solution. Even under these circumstances the organs are not easy to find on account of their transparency. This difficulty may be minimized by vitally staining the preparation with methylene blue, a treatment that stains the prothoracic glands more or less intensely without impairing their viability in physiological experiments.

The following description is based on numerous dissections of the Cecropia silkworm and especially of the diapausing pupa of this species:

As shown in Figure 1, the prothoracic glands are bilaterally placed in the pro- and mesothorax. Each gland consists of a main mass and a number of branches. The main mass lies immediately internal to the large tracheae at the level of the prothoracic spiracle. Branches radiate from this main mass, as indicated in Figure 1. The branch extending in the direction of the brain is short and compact, but the other branches consist, for the most part, of fusiform or stellate cells strung together on tenuous, intercellular bridges. In the region of the mesothoracic spiracle the cells of the gland show a second concentration. All of these branches are laterally placed in the insect and are covered and partially entwined with fat-body. The gland is richly supplied with nerves and tracheoles.

The microscopic structure of the gland is indicated in Figures 2 and 3. Little internal structure is visible in the living gland (Fig. 2) and greater detail is not obtained after fixation and staining with most agents. In my experience, best results are obtained by fixation in Helly's solution and staining with haematoxylin. As shown in Figure 3, the gland is then found to consist of enormous, complexly folded nuclei surrounded by a scanty, cytoplasmic syncytium. Each nucleus contains a single, prominent nucleolus.

Although the above description applies, specifically, to the diapausing pupa, the prothoracic glands show essentially the same arrangement in the later larval instars. With the onset of adult development, dissection becomes very difficult, but the glands are still present and seem to assume a more racemose configuration in the region of the prothoracic spiracles. I have not succeeded in demonstrating the organs in the adult and believe them to be absent. It is probable that a description of the glands in young larvae, in developing pupae, and in the adult (?) must await reconstruction from serial sections.

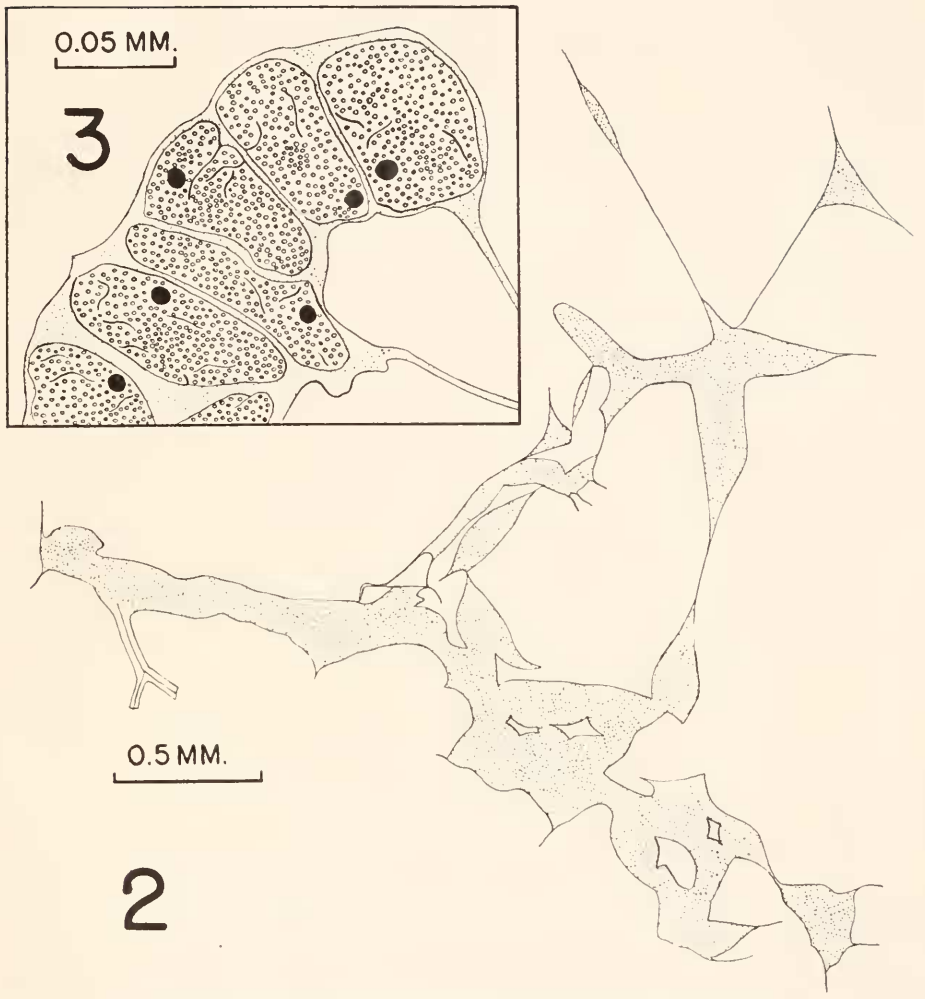

FIGURE 2. Living fragment of a prothoracic gland of a diapausing Cecropia pupa, showing branching and anastomosing structure

FIGURE 3. Highly magnified view of a fragment of a prothoracic gland of a diapausing Cecropia pupa. Fixed in Helly's solution and stained with Delafield's haematoxylin.

DISCUSSION

It is not difficult to understand why prothoracic glands were overlooked for so many years. For, in the species that I have examined, the gland is a tissue rather than a compact organ. I regard complete or even partial excision of the prothoracic glands as technically incompatible with the survival of the animal. To secure individuals devoid of prothoracic glands, one must necessarily work with isolated abdomens.

Prothoracic glands are widely distributed among the Lepidoptera and, most probably, occur in all members of the Order (Lee, 1948). Preliminary, gross dis-

section has failed to reveal their presence in larvae of Diptera, Coleoptera, and Trichoptera, but they are conspicuous in the hymenopterous larva of the saw-fly, *Cimbex americana.*

Of special interest is the description by Pflugfelder (1938) of a bilateral glandular cell complex in the head region of the walking-stick, *Dixippus.* This complex was termed the "ventral head gland of inner secretion." It arises as an epithelial invagination in the region of the head and apparently extends into the prothorax where it is attached to the muscles and tracheae of this region. It attains maximal size just before the last moult, at which time the nuclei become lobulated. After the last moult the nuclear membrane is lost and the chromatin lies irregularly in the cytoplasm. The gland is degenerate in the adult. Pflugfelder suggested that the gland might play a role in nymphal development, but considered its extirpation to be impossible.

On the basis of this description, I propose that the ventral head glands of Pflugfelder are homologous with the prothoracic glands of Lepidoptera. If this is confirmed, then prothoracic glands must be present in certain hemimetabolous as well as holometabolous insects and the endocrinology of insect development must be re-examined from this point of view.

A careful survey of all Orders is required. Since the gland may conceivably vary in its structure and position, such a search should be directed towards a study of embryos with special reference to the presence and fate of invaginations of the lateral part of the second maxillary segment.

Summary

1. The data relative to the thoracic control of insect development are surveyed. Embryonic development seems to be controlled by a thoracic center in all insects. Postembryonic development seems to be controlled by a thoracic center in at least the Lepidoptera and the Megaloptera.

2. In the Lepidoptera the thoracic center for postembryonic development can be identified as the "prothoracic gland." A review is presented of the literature describing these organs.

3. The thoracic center for embryonic development coincides with the position occupied by the prothoracic glands in lepidopterous embryos. It is therefore possible that the prothoracic glands may function as the embryonic differentiation center in at least certain species.

4. The morphology and histology of the prothoracic glands are described in the Cecropia silkworm. Homologous organs are present in saw-fly larvae and most probably in the walking-stick, *Dixippus.*

5. Since prothoracic glands, or their homologues, may be widely distributed in embryonic and postembryonic stages of insects, these organs present special physiological interest and merit further study.

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